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# Abiotic Stress in Plants

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/55865>

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## 1. Introduction

Living on the same planet, plants means a lot to us. No matter being taken, as our food or treated with great commercial significance, plants are so indispensable that we have to learn how to protect, make use of, and most important of all, get on well with them. In the first place, what we all understand is: plants are distinguished from us or other animals by being unable to escape from the surrounding circumstances. Thus, when they are confronted with living-threaten pressures, their only choice is to try their best to adjust to them.

The second is "But how?" Plants have developed plenty of physical and biochemical strategies to face up to adverse conditions. Fortunately, thanks to so many excellent researchers' efforts in this field, we have been making so many progresses in identifying and characterizing the mechanisms on how plants perceive outside stress and response to it. Unfortunately, that's far from enough. In this chapter, we will mainly discuss abiotic stress and endeavor to elucidate the mechanism of various reactions plants take at the molecular level.

Before we start our discussion, we probably need to know what abiotic stress is all about. Basically, it includes all the non-living environmental factors that can negatively or even harmfully affect the growth and productivity of plants. Commonly, we choose to put drought, flooding or submergence, salinity, extreme temperatures on our daily researching agenda due to their key roles in producing yield loss of agricultural or industrial crops worldwide. But other kind of abiotic stress is entitled to be paid more attention, such as high light, deficits of inorganic nutrients (nitrogen, phosphorus, potassium et al.), and for sure, they are of definite importance for plants' growth and development. Moreover, one factor we can not set aside is human behavior, which in a large sense put considerable pressure on plants. Residuals of chemicals brought by agricultural practice to improve yield may generate stress, and the increased modification of the atmosphere by human activities is gaining weight. And what we should really stress on is the compounding damaging effects

by multiple stress factors acting simultaneously. Thus for plants exposed in diverse stress conditions, struggling for surviving, they organically adapt a complicated interplay of signaling cascade to perceiving stress signal, then amplifying, transmitting, and finally triggering stress responses. Furthermore, there do exist overlap between different kinds of stress responses, which truly explains the cross-tolerance phenomenon, a measure taken by plants facing with combining stresses. Here, we are going to introduce signal transduction mechanisms in plants under stressful circumstances, hoping to give readers a general idea about how plants survive in different stressful situations.

## 2. Signal transduction

In general, for plant cells, signal transduction starts from the receptor activation, then the generation of second messengers translating the primary external signal to intracellular signals. These intracellular messengers will be further interpreted by their co-workers resulting in the inspiration of downstream pathways. During the whole process, reversible protein phosphorylation frequently happens; this can activate various transcription factors inducing the expression of stress responsive genes. Moreover, other components are also essential for the pathway to process. They have always been mentioned as signaling partners, mainly working in recruiting and assembling signal complexes, targeting signaling molecules, as well as controlling their lifespan.

Simply saying, the signal transduction pathway is a delicate cooperating process conducted by each single participator including receptors/sensors, second messengers, phosphoprotein cascades, transcription factors, and stress-responsive genes. Eventually the precise and optimal response will be triggered to protect plants from damages in a large sense. As far as we know, signal transduction is indispensable for many cellular activities and their coordination, and most of its steps are complicated occurring in a time and space-dependent manner. In the following part, we are going to explore every single step of signal transduction in order to understand how plants cope with various stress in their lifespan.

### 2.1. Sensors

#### 2.1.1. Complexity in researching on sensors

Sensors act as the molecules pioneering in perceiving stress stimulus and relaying the signal to downstream molecules to initiate the signal transduction pathway. As the first participator in the pathway, they must be of great researching meaning. However, they are also the mainly intricate role for us to recognize.

Firstly, most of the abiotic stress signals themselves are complicated which probably comprise several physical or chemical signals. Taken the cold stress as an example, it can induce both osmotic stress and mechanistic stress. Similarly, drought may be accompanied by osmotic stress, ionic stress, a mechanistic signal, and heat stress in some cases. Based on these facts, it is natural for us to deduce that for plants there probably exists inequality in

treating each single stimulus in accordance with the plant status or the stress severity. That is to say, a simple stimulus may diversely deliver complicated information to the plants, that's exactly why it is very likely for plants to have multiple cellular sensors to perceive each stress signal or one attribute of that signal. Secondly, the redundancy of signal perception make it even harder to identify and confirm each sensor relating to each stress stimulus, since knocking out one receptor may not significantly affect stress signaling outputs. Thirdly, even if we find a putative sensor, how to prove our hypothesis could also become a headache. Because different sensors probably vary in molecular identities, signal-perceiving modes, outputs, and also subcellular localizations, no wonder not much is known about plant abiotic stress sensors.

### 2.1.2. Putative sensors for perceiving stress signal

First of all, how can an external signal turn out to be internal? Where are the receptors/sensors and transporters? These will be the first bunch of questions we are going to ask. Imaging if we are plant cells, what will be the first weapon we use to maintain inner homeostasis when suffering from the outside disturbances? The answer will probably be "plasma membrane". So far, many researchers have demonstrated that the plasma membrane (PM) is responsible for perceiving and transmitting external stress signals, as well as responding to them. For example, when plants are under salinity stress, salt reaches the PM first, which makes the membrane lipids and transport proteins start to regulate permeability of this membrane triggering primary responses (Cooke and Burden, 1991). In many plants, changes in PM lipids, such as sterols and fatty acids, have been observed responding to salt stress and may contribute to the control of membrane fluidity and permeability, as a primary stress-responsive reaction (Elkahoui et al., 2004). Therefore, it is suggested that physical properties of membranes (lipid composition, fatty acid composition) may lead us to find potential sensors perceiving stress signals.

Secondly, let's stress a little bit more on the most common stress signals, cold, drought, and salinity. All of these three stresses have been detected to induce transient  $\text{Ca}^{2+}$  influx into the cell cytoplasm (Sanders D et al., 1999; Knight, 2000). Thus we can hypothesis channels responsible for this  $\text{Ca}^{2+}$  influx possibly acting as a sensor for these stress signals. Based on what we have discussed above, signaling reception may involve changes in membrane fluidity and cytoskeleton reorganization, which are also confirmed in early cold signaling (Sangwan et al., 2001; Wang and Nick, 2001). Coincidentally, cold-induced  $\text{Ca}^{2+}$  influx in plants occurs only after the occurrence of a rapid temperature drop (Plieth et al., 1999). Taken together, physical alterations in cellular structures may activate certain  $\text{Ca}^{2+}$  channels under cold stress, which indirectly suggests that  $\text{Ca}^{2+}$  channels might be a putative sensor.

Except the ion channel as a whole, other types of functional proteins can hardly be ignored on the list of sensors. So far, studies on plants and other systems have also identified several kinds of sensors. And in order to find sensors effectively in plant abiotic pathways, we need to borrow the experience and results of researches on other species. It is known that for plants, cold, drought, and salt stresses will all induce the accumulation of compatible osmolytes and antioxidants (Hasegawa et al., 2000). In yeast and in animals, mitogen-

activated protein kinase (MAPK) pathways are responsible for producing osmolytes and antioxidants, which are activated by receptors/sensors such as protein tyrosine kinases, G-protein coupled receptors, and two-component histidine kinases. For plants, only histidine kinases may have been explored and clarified in a deeper sense compared with others.

Retrospecting the history of histidine kinase, one important discovery is the cyanobacterium histidine kinase Hik33 (Suzuki et al., 2000) and the *Bacillus subtilis* histidine kinase DesK (Aguilar et al., 2001) being identified as thermosensors. Unfortunately, even if several putative two-component histidine kinases have been found in *Arabidopsis thaliana* (Urao et al., 2000), none of them can be confirmed as thermosensors. However in yeast, a two-component histidine kinase named SLN1 has been identified as a type of membrane protein sensor for osmotic stress perception (Maeda et al., 1994, 1995). And then later researches found out AtHK1, an *Arabidopsis* histidine kinase, can complement mutations of *SLN1*. Therefore AtHK1 may participate in osmotic stress signal transduction in plants (Urao et al., 1999). In conclusion, understanding the function of putative histidine kinases and their relationship with MAPK pathways not only help us dig out more sensors but also, even more important, find out how they work in signal transduction pathways.

Thirdly, in plants, the receptor-like kinases and G-protein are worthy to be mentioned in the searching for stress signal sensors. Why? The stress hormone abscisic acid (ABA) makes us study on them who may contain putative stress sensors. It is well-known that the ABA is of great significance in stress signaling, thus, to understand how ABA is perceived certainly will contribute to revealing the hidden sensing-processes of stress signals. Generally, the researches on ABA perception mechanisms always relate to putative receptor-linked components or those putative receptor molecules regulated by stress or ABA.

Here we are going to mention a different way of osmolyte production that involves the pathways triggering the activation of late embryogenesis-abundant (*LEA*)-type genes representing damage repair processes (Zhu, 2001; Xiong and Zhu, 2002). And these *LEA*-like genes under cold, drought, and salt stress are modulated by phosphoinositols who are closely connected with the activity of phospholipase C, which in plants might be regulated by G-proteins. Moreover evidences suggest G-protein coupled receptors may take part in perceiving a secondary signal derived from these stresses (Ullah et al., 2001; Wang et al., 2001), which may brings a hint that G-protein may have a position on primary sensors list.

On the other hand, *Arabidopsis* heterotrimeric G-protein subunit GPA1 may be a part of ABA response in guard cells but has no relation with ABA-induced stomata closure. Moreover GPA1 interacts with the G-protein couple receptor-like protein GCR1. Researches on *gpa1* mutants and *gcr1* mutants bring us much more information on finding receptors for ABA. Also some small G proteins are referred to as negative regulator of ABA responses in *Arabidopsis*, like ROP10. But the really surprising discovery comes into the world in 2009. In that year, two research groups from the USA and Germany reported in Science that they had identified a small protein family binding to ABA interacts with ABA Insensitive 1 and 2 (*ABI1* and *ABI2*), two type 2C protein phosphatases (PP2Cs). And they are negative regulators of ABA signaling (Ma et al. 2009, Park et al. 2009).

With the proceeding of the pathway, we can see that abiotic stresses also give birth to second signaling molecules (discussed below). Therefore, in the next part, we are going to pay attention to the second messengers and their performance in signal transduction pathways.

## 2.2. Second messengers

Several second messengers are active participators in stress signal transduction. Mainly they are groups of small intracellular signaling molecules or ions, normally locating in the cytoplasm of a cell and responding to a signal received by a cell-surface sensor, which activates various kinases to regulate other enzymes' activities. What we mention frequently as second messengers are reactive oxygen species, lipid phosphates-derived signals, and cyclic nucleotides-related signals. Besides, some plant hormones also work as secondary signal molecules under stress conditions.

### 2.2.1. Reactive oxygen species (ROS)

ROS are species of oxygen which are in a more reactive state than molecular oxygen, resulting from excitation or incomplete reduction of molecular oxygen. Generally, ROS contains both free radical ( $O_2^{\bullet-}$ ,  $RO^{\bullet}$ ,  $HO_2^{\bullet}$ ,  $OH^{\bullet}$ ), and non-radical forms ( $H_2O_2$ ,  $^1O_2$ ). For plants, they tend to be a two-edged weapon. On one hand, they are highly reactive and toxic, always taken as unwelcome harmful by-products of normal cellular metabolism, and causes damage to proteins, lipids, carbohydrates, DNA which ultimately results in cell death in plants. On the other hand, it has also been proved that ROS can affect genes' expression and signal transduction pathways, which mean that cells may use it as biological stimuli and signals to activate and regulate various genetic stress-response processes (Foyer and Noctor 2009).

Since it means a lot to plants' life, where and how it can be produced? In photosynthetic tissues, the chloroplast is the prime source of ROS. But for the non-photosynthetic tissues, mitochondria are the leader in production. In chloroplasts, photosystem I and II (PSI and PSII) are the major sites for the production of  $^1O_2$  and  $O_2^{\bullet-}$ . In mitochondria, complex I, ubiquinone and complex III of electron transport chain (ETC) are the major sites for the generation of  $O_2^{\bullet-}$ . In addition to the mitochondria and NADPH oxidases, additional cellular sources of ROS production include a host of other intracellular enzymes such as xanthine oxidase, cyclo-oxygenases, cytochrome p450 enzymes, and lip-oxygenases for which oxidants act as part of their normal enzymatic function.

Consequently, when plant cells are under stresses, the rate of ROS production usually goes up, inspiring the activities of antioxidants and scavenging enzymes to keep plants live a healthy life. Fortunately, plant cells possess very efficient enzymatic (superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX; glutathione reductase, GR; monodehydroascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR; glutathione peroxidase, GPX; guaiacol peroxidase, GOPX and glutathione-S- transferase, GST) and non-enzymatic (ascorbic acid, ASH; praline; glutathione, GSH; phenolic

compounds, alkaloids, non-protein amino acids and  $\alpha$ -tocopherols) antioxidant defense systems cooperatively working on controlling the cascades of uncontrolled oxidation and protecting plant cells from oxidative damage by scavenging of ROS. Eventually, the equilibrium has maintained between ROS production and antioxidant defense systems.

However, this balance will always be perturbed by various biotic and abiotic stress factors such as salinity, UV radiation, drought, heavy metals, temperature extremes, nutrient deficiency, air pollution, herbicides and pathogen attacks. Once it has been challenged, various signals pathways start to be proceeded to mediate the disturbances to protect cells from harm brought by extra ROS. For example, when osmotic stress comes, various plant species show an obviously reduced assimilation rate due to stomatal closure (Huchzermeyer and Koyro 2005). This result can be owed to an excessive production of reactive oxygen species (ROS) who are highly destructive to lipids, nucleic acids, and proteins (Kant et al. 2006; Türkan and Demiral 2009; Geissler et al. 2010).

First and foremost, having been identified as second messengers how does ROS affect stress signal transduction? Several enzymes which are involved in cell signaling mechanisms are also potential targets of ROS. These include guanylyl cyclase (E. Vranova, S. Atichartpongkul, 2002), phospholipase C (C.H. Foyer, G. Noctor, 2003), phospholipase A2 (I.M. Moller, 2001) and phospholipase D (A.G. Rasmusson, K.L. Soole, 2004). Ion channels may be targets as well (G. Noctor, R.D. Paepe, 2006), among which calcium channels was mentioned (D.M. Rhoads, A.L. Umbach, 2006). Since calcium has ubiquitous functions in plant stress signal transduction pathway, we are interested in the relationship between ROS and calcium. Before dive into calcium, let's back to NADPH oxidases that are an important ROS-generating system. RBOHs shorting for respiratory burst oxidase homologs is always an eye-catching topic. Recent evidence points out RBOHs relate to heavy-metal induced accumulation of ROS (Pourrut et al. 2008) and early response to salt stress (Leshem et al. 2007). Subsequently, ROS produced by Rbohs are thought to activate  $\text{Ca}^{2+}$  channels leading to further increases in cytosolic  $\text{Ca}^{2+}$  (Foreman et al. 2003) and downstream signaling. In general, it has been suggested that ROS took part in the regeneration of  $\text{Ca}^{2+}$  signals by activating  $\text{Ca}^{2+}$  channels. Then additional signal transduction was triggered through  $\text{Ca}^{2+}$ -mediated pathways (reviewed in Mori, I.C. and Schroeder, J.I. 2004).

Except the interaction with calcium, another route for ROS to work is that ROS themselves can directly modify signaling molecules through redox regulation. Redox status inside a cell is essential to the correct functioning of many enzymes, which can be used to alter enzyme activity; thus alteration of the redox status could be treated as a signaling mechanism (Gamaley and Klyubin, 1999). One of the most important and well-known redox-sensitive molecules in this respect is glutathione (GSH), which can form the GSH/GSSG couple. The balance between the GSH and GSSG takes the central position to maintain cellular redox state (C.H. Foyer, G. Noctor, 2005). But ROS like  $\text{H}_2\text{O}_2$  can affect the process of lowering the cells' GSH content to alter the redox status. Meanwhile, it also has been suggested that enzymes such as ribonucleotide reductase and thioredoxin reductase, as well as transcription factors, might be among the targets for altered redox status. In detail, cysteine

residues of molecules may be key active sites as targets for redox regulation. And these molecules can act as potential sensors for ROS (Xiong, L. and Zhu, J.K. 2002).

Secondly, ROS are very likely to play a significant role in the activation of stress-responsive genes, especially those who encode enzymes responsible for antioxidants biosynthesis or enzymes directly detoxify reactive oxidative radicals. For example,  $H_2O_2$  production is thought to be raised under various abiotic stresses, which can enhance gene expression of active oxygen scavenging (AOS) enzymes. NO, produced under salt stress, could serve as a second messenger for the induction of PM H-ATPase genes' expression, which promote PM H-ATPase activity (Liqun Zhao, Feng Zhang et al., 2004).

Thirdly, we are going to stress a little more on  $H_2O_2$  and NO. In maize,  $H_2O_2$  production grows up induced by chilling stress, and exogenously applied  $H_2O_2$  lifted up chilling tolerance (T.K. Prasad, M.D. Anderson, 1994). Increased  $H_2O_2$  production has been detected occurring gradually responding to salt stress in rice plants (N.M. Fadzilla, R.P. Finch, 1997). Moreover,  $H_2O_2$  was also reported to induce small heat shock proteins (HSP26) in tomato and rice (J. Liu, M. Shono, 1999; B.H. Lee, S.H. Won, H.S. Lee, 2000). However, it was recently shown that  $H_2O_2$  produced by apoplastic polyamine oxidase can influence the salinity stress signaling in tobacco and can play a role in balancing the plant response between stress tolerance and cell death (Moschou et al. 2008). NO has also been suggested to act as a signal molecular mediating responses to biotic and abiotic stresses. Under salt stress, NO could serve as a second messenger for the induction of PM H-ATPase expression, which may account for the enhanced PM H-ATPase activity. Thus, ion homeostasis is reestablished so as to adapt to salt stress (Liqun Zhao, Feng Zhang et al., 2004).

Furthermore, researches on ABA give us much more information on  $H_2O_2$  and NO in signal transduction. So let's take a look at how they work in ABA signaling and other signal transduction pathways. The process of stomata closure regulated by ABA in a large sense require the generation of  $H_2O_2$ . Moreover,  $H_2O_2$  production may be a prerequisite for ABA-induced stomatal closure (Zhang, X., Zhang, L. et al. 2001). Experiments have found out mutations in genes encoding catalytic subunits of NADPH oxidase, known as the major source for  $H_2O_2$  production, will impair ABA-induced ROS production, as well as the activation of guard cell  $Ca^{2+}$  channels and stomata closure (Kwak J.M., Mori, I.C. et al. 2003). In plants, both nitrate reductases and NO synthases (NOS) can contribute to NO generation. Loss-of-function mutations in Arabidopsis NOS, AtNOS1, impair ABA-induced NO production and stomata closure (Guo, F.Q., Okamoto, M. and Crawford, N.M., 2003).

On the other hand, accumulated evidence indicate ROS seem to play a central role in regulating Mitogen-activated protein kinase (MAPK or MPK) cascades (discussed later in detail). However, only the functions of MPK4, MPK3 and MPK6, out of the 20 Arabidopsis MAPKs, have been thoroughly characterized. What really counts is they can all be activated by ROS and abiotic stress. In addition, activities of MPK1 and MPK2 have been shown to be provoked by  $H_2O_2$  and ABA (Ortiz-Masia et al. 2007). Also MPK7 was found to be activated by  $H_2O_2$  under specific circumstances (Dóczy et al. 2007). Furthermore, the authors found that  $H_2O_2$  may probably have a generally stabilizing impact on MAPKKs (MAP kinase

kinases). In the future, we still have lots of work to elucidate how ROS regulate MAPK signaling in abiotic stress field.

All in all, even if we are holding a lot of evidence about the functions of ROS in abiotic stress, we still have to face up to those obscure steps relating to different mechanisms, not to mention hundreds of stress responsive genes involved in.

### 2.2.2. *Lipid-derived signal messengers*

It is well-known that cellular membranes contains a wide range of different lipids, including sphingo-, neutral-, glyco-, and phospholipids, all with unique biophysical properties. Beyond the structural role, some of them are equipped with direct signal-transducing properties. What we discussed in the sensors part is that membrane lipids can directly response to abiotic stress stimuli by modulating membrane fluidity or its other physiochemical properties, but in this part we will take another angle to demonstrate its significant function in the process of generating intracellular signaling molecules. Moreover lipids and their biogenesis and degradation enzymes play many direct or indirect roles to regulate or affect signaling and stress tolerance. In signal transduction, signaling lipids are distinguished for their low abundance and rapid turnover. They are rapidly formed responding to diverse stimuli through lipid kinases or phospholipases' activation. Thanks to the lipid-binding domains, these lipid signals can activate enzymes or recruit proteins to membranes leading to the activation of downstream signaling pathways resulting in specific cellular events and physiological responses. Studies on them find out, for plants, lipid signaling form a complex regulatory network responding to abiotic stress.

Basically, in eukaryotes, typical signaling lipids includes phosphatidylinositol lipids (polyphosphoinositides; PPIs), certain lyso-phospholipids, diacylglycerol (DAG), and phosphatidic acid (PA) (Munnik and Testerink, 2009; Xue et al., 2009; Munnik and Vermeer, 2010). Among them, PA is of great importance as a lipid second messenger in plants involved in various biotic and abiotic stress conditions. Based on thousands of researches, it is easy to detect almost every environmental cue can trigger a rapid PA response (Testerink and Munnik, 2005; Arisz et al., 2009; Li et al., 2009; Mishkind et al., 2009). How can PA be produced? Two ways in brief. Directly PA is generated through activation of phospholipase D (PLD), and indirectly a phospholipase C/diacylglycerol kinase (PLC/DGK) pathway regulated by two types of PLC enzyme named as the PI-PLCs (phosphoinositide-PLCs) and NPCs (non-specific PLCs). After the rapidly bounce up under stress, PA level will go back to normal when stimuli disappear. (Christa Testerink et al. 2011).

In most of the osmotic stress cases, both PLC/DGK and PLD pathways are activated leading to fast and transient PA accumulation, but exceptions also exist (Zonia and Munnik, 2004; Darwish et al., 2009; Hong et al., 2010). Besides responding to osmotic stress, we also see the PLD $\alpha$ 1 enzyme participating in cold, frost, and wound stress signaling (Bargmann et al., 2009; Hong et al., 2010) and probably by promoting responses to ABA, especially in stomata (Mishra et al., 2006). On the other side, PLC/DGK pathways also get activated by salinity (Arisz, 2010). Earlier we knew that AtPLC1, one of the PI-PLCs, was shown to be induced by salinity and drought (Hirayama et al., 1995), which is necessary for ABA-induced inhibition

of germination and gene expression (Sanchez and Chua, 2001). Recently, NPC4 (a NPC isoform) was found to modulate responses to ABA and bring enhanced salt and drought tolerance (Peters et al., 2010). In addition, several ABA signaling proteins have been identified as potential PA targets (Mishra et al., 2006), which has further suggested PA could mediate ABA responses. Meanwhile, cooperation between the NADPH oxidase isoforms RbohD, RbohF and PA brings more information for us to understand PA's function in ABA-induced ROS generation and stomatal closure (Zhang et al., 2009). Furthermore, PA also targets other protein kinases like SnRK2 protein kinase (Testerink et al., 2004), MAPK isoform MPK6 (Yu et al., 2010), sphingosine kinase (SPHK) (Liang Guo, XueminWang, 2012) to influence diverse signaling transduction pathways.

Other two important second messenger molecules - inositol phosphates Ins(1,4,5)P<sub>3</sub> and DAG (diacylglycerol) are worthy to be mentioned here. Firstly, InsP<sub>3</sub> was shown to release Ca<sup>2+</sup> from an intracellular store in the early 90s, but recently evidences pops up suggesting that InsP<sub>6</sub> was shown to release Ca<sup>2+</sup> at a 10-fold lower concentration than InsP<sub>3</sub> (Lemtiri-Chlieh et al., 2003; Teun Munnik, 2009), who can be generated by phosphorylating InsP<sub>3</sub>. By the way data can be found demonstrating that whole-plant IP<sub>3</sub> level goes up significantly within 1 min after stimuli occur, and keep the tendency for more than 30 min under stress. Under osmotic stress, in *Arabidopsis*, phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P<sub>2</sub>) is hydrolyzed to IP<sub>3</sub>. And IP<sub>3</sub> accumulation occurs coincidentally in a time frame similar to stress-induced calcium mobilization (Daryll B. DeWald et al., 2001). In conclusion, what we can say is under different stress, to identify the most critical second messenger molecules depends on the research on the network consisting of multiple polyphosphoinositides.

Secondly, diacylglycerol (DAG) is an important class of cellular lipid messengers, but for its function in plants, data is not sufficiently provided. In *Arabidopsis thaliana*, knocking out NPC4 results in DAG level decrease and compromises plant response to ABA and hyperosmotic stresses. On the other hand, overexpressing NPC4 leads to higher sensitivity to ABA and stronger tolerance to hyperosmotic stress than wild-type. And later experiments indicate that NPC4-produced DAG is converted to PA and NPC4 might be a positive regulator in ABA response and promote plant tolerance to drought and salt stresses (Carlotta Peters et al, 2010). Furthermore, all higher plant genomes sequenced so far lack both InsP<sub>3</sub> receptor and the DAG target, PKC (Munnik & Testerink 2009). In conclusion, we have reasons to believe in that PA rather than DAG are more likely to play a central role in stress signaling transduction.

At last, we'd love to say more about other types of phospholipases like secreted phospholipase A<sub>2</sub> and patatin-related phospholipase A (pPLA) who were shown to have functions in auxin signal transduction by cooperating with auxin receptors ABP1 or TIR1. (Günther F. E. Scherer et al., 2012 ). And this fact helps us to further confirm the significance of phytohormones in signaling transduction which will be discussed below.

### 2.2.3. Phytohormones

When it comes to phytohormones, strictly speaking, we can not conclude them as second messengers, but the first and foremost idea needs to be posted here is "The most powerful

players in intercellular regulation are plant hormones." (Wolfgang Busch, Philip N. Benfey, 2010). Owing to the broad and diverse functions, many of phytohormones were discovered before the dawn of molecular genetics (Sachs and Thimann, 1967; Thimann and Skoog, 1933). In generation, they are a large bunch of trace amount growth regulators, the best-known group comprises auxin (IAA), cytokinin (CK), gibberellic acid (GA), abscisic acid (ABA), jasmonic acid (JA), ethylene (ET), salicylic acid (SA), but the name list is growing by time. Here we add brassinosteroids (BR), nitric oxide (NO), polyamines, and strigolactone (SL). Indubitably phytohormones have various functions in growth and development. Indeed, they play central roles in nutrient allocation, and source/sink transitions. However based on former description relating to sensors and second messengers, we have to focus on here is that these low-molecular-weight compounds are indispensable for coordinating various signal transduction pathways during responses to various abiotic stresses.

Firstly, they function as systemic signals that can transmit information over large distances. Like ABA, it can be transported and play physiological roles at sites far away from where it is synthesized (Sauter, A. et al., 2001). And different types of cells have their own understanding even for the same hormones signal. And information from diverse hormones always triggers coherent responses of cells. This is signal perception at cellular level. Lucky for us, modern transcriptome profiling technologies have provided a global view of hormones' effects at the molecular level and identified hundreds to thousands of genes, the expression levels of which are modified by individual hormones (Goda et al., 2008). A large number of data have proved that treating plants with exogenous hormones will rapidly and transiently alter genome-wide transcript profiles (Chapman and Estelle 2009).

Secondly, complex networks of gene regulation by phytohormones under abiotic stresses involve various *cis*- or *trans*-acting elements. Some of the transcription factors regulated by phytohormones include ARF, AREB/ABF, DREB, MYC/MYB, NAC, WRKY and other key components functioning in signaling pathways of phytohormones under abiotic stresses will be briefly mentioned later. And they often rapidly alter gene expression by inducing or preventing the degradation of transcriptional regulators via the ubiquitin–proteasome system (Santner A, Estelle M, 2010).

Thirdly, the ability of plants to a wide range of environmental stresses is also finely balanced through the interaction of hormonal plant growth regulators and the redox signaling hub. Plant hormones produce reactive oxygen species (ROS) as second messengers in signaling cascades that convey information concerning changes in hormone concentrations and/or sensitivity to mediate a whole range of adaptive responses (Carlos G. Bartoli et al., 2012). For example, Brassinosteroids (BRs) can induce plant tolerance to diverse abiotic stresses by triggering H<sub>2</sub>O<sub>2</sub> generation in cucumber leaves (Cui et al., 2011). In the following part we will simply introduce how phytohormones work in signal transduction, and how they talk with each other when they exchange information.

Let's start from ABA, whose synthesis is one of the fastest responses to abiotic stress for plants. Under water stress, ABA synthesis triggers ABA-inducible gene expression leading to stomatal closure, thereby reducing water loss through transpiration, and consequently, a

reduced growth rate (Schroeder, J.I. et al., 2001). ABA also plays a vital role in adapting to cold temperatures. Cold stress induces the synthesis of ABA and the exogenous application of ABA enhances the cold tolerance of plants. A large number of genes associated with ABA biosynthesis and ABA receptors-encoding genes and downstream signal relays have been characterized in *Arabidopsis thaliana* (reviewed by Cutler SR et al., 2010). ABA activates the expression of many stress-responsive genes independently or synergistically with stresses, which makes it become the most studied stress-responsive hormone.

Other hormones, in particular CK, SA, ET, and JA, also have substantial direct or indirect performances in abiotic stress responses. CK is an antagonist to ABA, and under water shortage situation, CK levels usually decrease. But transgenic tomato rootstocks expressing IPT (isopentenyl transferase, a gene encoding a key step in CK biosynthesis) had improved root CK synthesis shown raised salinity stress tolerance (Ghanem ME et al., 2011). Meanwhile, by checking public microarray expression data for *A. Thaliana*, numerous genes encoding proteins associated with CK signaling pathways have been found affected by various abiotic stresses (Argueso CT et al., 2009). Although auxins, GAs, and CKs have been implicated primarily in developmental processes in plants, they regulate responses to stress or coordinate growth under stress conditions (Günther F. E. Scherer et al., 2012; F. Eyidogan et al., 2012). Auxins taking part in drought tolerance was postulated by researchers (ZhangS-W et al., 2009). What's more, BR was reported (mainly researches on exogenous application of BR) to induce stress-related genes' expression, which results in the maintenance of photosynthesis activity, the activation of antioxidant enzymes, the accumulation of osmoprotectants, and the induction of other hormone responses (Divi UK et al., 2009). In conclusion, there do exist a complex network for phytohormones to contribute to stress-induced reactions for plants. And due to the overlap between hormone-regulated gene suites in the adaptive responses, we have to discuss cross-talk between the different hormone signaling pathways as a extensive part of that complex network.

Earlier, it was reported that ABA can inhibit the biosynthesis of ethylene and may also potentially reduce the sensitivity of plants to ethylene (Sharp, R.E., 2002). Recently the expression of many other genes associated with auxin synthesis, perception, and action has been shown to be regulated by ethylene (Stepanova AN, Alonso JM, 2009). And it is not surprising that auxin has been found involved in ethylene biosynthesis very early. Meanwhile, CK was shown to be a positive regulator of auxin biosynthesis (Jones B et al., 2010).

Furthermore, GA and BR regulate many common physiological processes like the growth and development in rice seedlings (Wang L et al., 2009). Except BR, GA has another partner - SA. Transgenic *A. thaliana* plants constitutively overexpressing a GA-responsive gene became more tolerant under abiotic stress and this stronger tolerance was correlated with increased endogenous levels of SA (Alonso-Ramirez A et al., 2009).

Discussed above, ABA can regulate stomatal actions under stress conditions; however, it is not alone in that process. CK, ET, BR, JA, SA, and NO also affect stomatal function (reviewed by Acharya B, Assmann S, 2009). In detail, ABA, BR, SA, JA, and NO induce

stomatal closure, CK and IAA promote stomatal opening. And we mentioned before, NO acts as a key intermediate in the ABA-mediated signaling network in stomatal closure. Moreover BR-mediated signaling was regulated by ABA, and in turn, ABA was also shown to inhibit BR-induced responses under abiotic stress (Divi U et al., 2010). And it is not hard to deduce that there are other tricky relationships between different hormone-involved pathways. Cross-talk between the phytohormones results in synergetic or antagonist interactions, which is crucial for plants in abiotic stress responses. To characterize the molecular mechanisms regulating hormone synthesis, signaling, and action means a lot to modificate hormone biosynthetic pathways to develop transgenic crop plants with promoted tolerance to abiotic stress.

### 2.3. $\text{Ca}^{2+}$ as an intermediate signal molecule

So far, we have already touched some grounds related to Calcium ( $\text{Ca}^{2+}$ ) functioning in signal transduction. Among all ions in eukaryotic organisms, it is likely to be the most versatile one who almost links to all aspects of plant development, not to mention many regulatory processes. The reason why it is so powerful may root in its flexibility in exhibiting different coordination numbers and complex geometries, and this ability makes it easily form complexes with proteins, membranes, and organic acids. However we won't detect high cytosolic or organelle  $\text{Ca}^{2+}$  concentrations resulting from the tight management of various  $\text{Ca}^{2+}$  pumps and transporters. The reason why the concentration needs to be controlled is that higher  $\text{Ca}^{2+}$  concentrations can chelate negatively charged molecules in the cell leading to cytotoxicity. Interestingly, all the secondary signaling molecules we mentioned above may activate transient increases in cytosolic  $\text{Ca}^{2+}$ , and transient elevations in cytosolic  $\text{Ca}^{2+}$  concentration have been documented to have relationship with a multitude of physiological processes linking to abiotic stress responses. So we may wonder concentration control probably will help us tell the story of another famous second messenger---  $\text{Ca}^{2+}$  in signal transduction for plants under abiotic stress.

Earlier in 1982, research on the green algae *Chara* told us the cytosolic  $\text{Ca}^{2+}$  concentration change predicted  $\text{Ca}^{2+}$  might work as a second messenger in plants (Williamson and Ashley, 1982). Based on later reports, it has been found various stimuli will spur their own special  $\text{Ca}^{2+}$  responses differing in where and how changes happen (Johnson et al., 1995; Tracy et al., 2008), which exactly supports the former concept of  $\text{Ca}^{2+}$  signature. For plants, to maintain  $\text{Ca}^{2+}$  homeostasis, they need the help from  $\text{Ca}^{2+}$  channels, pumps, and exchangers (carriers) to make specific adaptation to every kind of stimulus (Kudla et al., 2010). Later, cellular  $\text{Ca}^{2+}$  signals are decoded and transmitted by  $\text{Ca}^{2+}$ -binding proteins that relay this information into downstream responses. Major  $\text{Ca}^{2+}$  signal transduction routes contain  $\text{Ca}^{2+}$ -regulated kinases mediating phosphorylation events and regulation of gene expression via  $\text{Ca}^{2+}$ -regulated transcription factors and  $\text{Ca}^{2+}$ -responsive promoter elements.

Generally speaking,  $\text{Ca}^{2+}$  signaling comprises three phases: generation of a  $\text{Ca}^{2+}$  signature, sensing the signature and transduction of the signal (Reddy and Reddy, 2004). Having discussed above, we are informed that the concentration change are always triggered by

cellular second messengers, such as NAADP, IP<sub>3</sub>, IP<sub>6</sub>, Sphingosine-1-Phosphate, and cADPR (Allen and Sanders, 1995; Navazio et al., 2000; Lemtiri-Chlieh et al., 2003). Then a specific cellular Ca<sup>2+</sup> signature is sensed by Ca<sup>2+</sup>-binding proteins, the Ca<sup>2+</sup> sensors (Dodd et al., 2010; Reddy and Reddy, 2004). The sensors themselves may become active to transduce the signal by themselves, or choose to bind to their interacting proteins and affect their partners' activity to transduce the signal. In detail, there are three major classes of Ca<sup>2+</sup> sensors identified in plants. The first one is calmodulins (CaMs) and calmodulin-related proteins (CMLs). CaMs is a group of small acidic protein, highly conserved in eukaryotes (Snedden and Fromm, 2001), and contains four EF hands (one major Ca<sup>2+</sup> binding motif) where bind Ca<sup>2+</sup>. Moreover this binding action induces a conformational change of CaM, leading to exposure of hydrophobic surfaces and further triggering electrostatic interactions with target proteins - CaM-binding proteins (CBPs) (Hoeflich and Ikura, 2002). CBPs have been found to take part in regulating transcription, metabolism, ion transport, protein folding, cytoskeleton-associated functions, protein phosphorylation and dephosphorylation, as well as phospholipid metabolism (Yang and Poovaiah, 2003; Reddy and Reddy, 2004). Furthermore, different CaM proteins exhibit differential expression and are likely to show differential affinity to Ca<sup>2+</sup> and to their target proteins (McCormack et al., 2005; Popescu et al., 2007), which makes CaM be equipped with multiple capabilities in Ca<sup>2+</sup> signal transduction. With many similarities to CaMs, CMLs are mostly composed of four EF-hands and lack other known functional domains. Like CaMs, they relay the signal by binding to other proteins resulting in activation or inactivation of interacting proteins. Over 300 proteins that interact with CaMs and CMLs have been identified in plants (Popescu et al., 2007). The second class of Ca<sup>2+</sup> sensor is represented by the calcium-dependent protein kinases (CDPKs/CPKs) who are serine/threonine protein kinases contain a catalytic kinase domain and EF-hand motifs (Cheng et al., 2002). The third typical sensor type is the EF-hand-containing Ca<sup>2+</sup>-modulated protein named SCaBP (SOS3 (Salt-Overly-Sensitive 3)-like Ca<sup>2+</sup>-binding proteins)/Calcineurin B-like (CBL) proteins, which is plant-specific (Luan et al., 2002). CBLs interact with a family of protein kinases called CBL-interacting protein kinases (CIPKs) (Luan et al., 2009; Weinl and Kudla, 2009; Batistic et al., 2010). In addition to EF-hand-containing Ca<sup>2+</sup> binding proteins, there are other proteins without that motif acting as sensors who also can bind Ca<sup>2+</sup>, like PLD (introduced in 2.2.2), annexins and C2 domain-containing proteins (Clark and Roux, 1995; Reddy and Reddy, 2004; Laohavisit and Davies, 2011), however their functions in abiotic stress responses haven't been deeply explored, and only some reports suggest PLD and annexin be relevant to stress signal transduction (White et al., 2002; reviewed by Laohavisit and Davies, 2011).

However here we will mainly stress on the EF-hand-containing sensors due to their considerable significance in signal transduction pathways. Based on their functional styles, these sensors are assigned into two camps termed as sensor relays and sensor responders (Kudla et al., 2010). The sensor relays do not have any known enzymatic or other functional domains except the EF hands. They interact with other proteins and regulate their activities, just like CaMs/CMLs, and CBLs (with one exception, CaM7) (McCormack et al., 2005; Luan, 2009; DeFalco et al., 2010). The members of another camp are characterized by an additional a catalytic or functional domain, except EF hands, whose activity is regulated by Ca<sup>2+</sup> binding to EF-hand motifs. So definitely CDPKs belong to this camp, and other members are

$\text{Ca}^{2+}$ - and  $\text{Ca}^{2+}$ /CaM-dependent protein kinases (CCaMKs), some DNA or lipid binding proteins, and a few enzymes (Day et al., 2002; Yang and Poovaiah, 2003; Harper and Harmon, 2005). Many calcium sensors are coded by multiple genes, and expression of many of these is induced by stresses (DeFalco et al., 2010).

Then, let's take a look at what will be the next move of those  $\text{Ca}^{2+}$ -activated sensors. Firstly, they can directly bind to *cis*-elements in the promoters of specific genes and induce or repress their expression. Secondly, they will choose to bind to DNA binding proteins and activate or inactivate them, thereby resulting in activation or repression of gene expression. The third path belongs to the activated  $\text{Ca}^{2+}$ -regulated protein kinases (CDPK, CaM binding protein kinase (CBK), CIPK and CCaMK) or phosphatases. They phosphorylate/dephosphorylate a transcription factor (TF), respectively, resulting in activation or repression of transcription, which allow for the perception and transmission of  $\text{Ca}^{2+}$  signatures directly into phosphorylation cascades that orchestrate downstream signaling responses (Weinl and Kudla, 2009). The most well-known TFs involved in the phosphorylation include Calmodulin binding transcription activators (CAMTAs; also referred to as signal-responsive proteins or ethylene-induced CaM binding proteins) (Reddy et al., 2000), MYB family (Popescu et al., 2007), the WRKY family (Park et al., 2005; Popescu et al., 2007), basic leucine zipper (bZIP) TFs, like TGA3 (Szymanski et al., 1996) and ABA-responsive TFs ABF1, 2, 3, and 4 (reviewed in Galon et al., 2010), and CBP60s who is a plant-specific CaM binding proteins family (Reddy et al., 1993; Zhang et al., 2010), as well as members of NAC family (Kim et al., 2007; Yoon et al., 2008). However they are TFs binding to  $\text{Ca}^{2+}$ /CaM under  $\text{Ca}^{2+}$  regulation, so we posted here another two TFs who can directly bind  $\text{Ca}^{2+}$ . One is encoded by *Arabidopsis* *NaCL-INDUCED GENE (NIG)* (Kim, J., and Kim, H.Y., 2006), and the other is At-CaM7 (Kushwaha et al., 2008). In sum,  $\text{Ca}^{2+}$  and their interacting proteins served as the upstream elements play an important role in regulating of some stress genes expression.

Meanwhile, what performance they will give in each specific abiotic stress condition needs to be simply introduced here. For drought stress, cellular  $\text{Ca}^{2+}$  transmits drought signals to regulate the physiological responses induced by drought stress (Dai et al., 2007). It has been found that  $\text{Ca}^{2+}$  treatment increased protection against membrane lipid peroxidation and stability of membranes and therefore resulted in the increase of drought resistance of rice seedlings. It is also reported that in wheat  $\text{Ca}^{2+}$  may reduce the adverse stress effects by elevating the content of proline and glycine betaine, thus improving the water status and growth of seedlings and minimizing the injury to membranes (Geisler et al., 2000; Munns et al., 2006; Goldgur et al., 2007). Additionally,  $\text{Ca}^{2+}$ /CaM means a lot to the process of ABA-induced drought signal transferring under PEG stress. And ABA synthesis correlates with cytoplasmic  $\text{Ca}^{2+}$  concentrations ( $[\text{Ca}^{2+}]_{\text{cyt}}$ ) (Rabbani et al., 2003; Noctor, 2006). We know how important ABA is to the stomatal status, and now more studies have established a close relationship between  $[\text{Ca}^{2+}]_{\text{cyt}}$  oscillation and stomatal status. In addition, in *Arabidopsis* genome, 9 SOS3 homologs (SCaBP/CBL) and 22 SOS2 homologs (SOS2-like protein kinases - PKS/CBL-interacting protein kinases-CIPK) were identified. By the way, SOS2 is a

serine/threonine protein kinase with an SNF1/AMPK-like catalytic domain and a unique regulatory domain (Liu et al., 2000). Individual SCaBP/CBL interacts with PKS/CIPK with different specificities (Gong et al., 2004; Luan et al., 2002). And it is indicated that the interaction between SCaBP5 and PKS3 may interpret  $\text{Ca}^{2+}$  signatures resulting from ABA or drought stress signals. On the other hand, SOS3 interact with and activate the SOS2, whose mutation also confers salt sensitivity. Then the activated SOS2 phosphorylates and regulates ion transporters such as the  $\text{Na}^+/\text{H}^+$  antiporter SOS1 controlling long-distance  $\text{Na}^+$  transport from the root to shoot, which eventually leads to the restoration of ion homeostasis in the cytoplasm under salt stress (Zhu, J.K., 2003).

In light of salt stress, like other stresses, it is perceived at cell membrane and then trigger intracellular-signaling cascade including the generation of secondary messenger molecules like  $\text{Ca}^{2+}$  and protons. For instance, it was found that in barley roots, under NaCl stress,  $\text{Ca}^{2+}$ -CaM system may work in activating tonoplast  $\text{H}^+$ -ATPase and regulating  $\text{Na}^+$  and  $\text{K}^+$  uptake with involvement of SOS signal transduction pathway (Brini et al., 2007). In Arabidopsis, experiments to overexpress AtCaMBP25 (a CaM binding protein) who may be a negative regulator of osmotic stress tolerance find the transgenic Arabidopsis plants show higher sensitivity to osmotic stress, while the antisense plants gain more tolerance under salt stress (Perruc, E. et al., 2004).

Furthermore, when it comes to uncomfortable temperature, is there any position for  $\text{Ca}^{2+}$ ? For sure. The  $\text{Ca}^{2+}$  channels have shown their power for the growth of root hairs and the low temperature acclimation of chilling-resistant plants. That's why we find data indicating that the activity and stability of  $\text{Ca}^{2+}$ -ATPase under 2 °C low temperature are the key factors in the development of cold resistance of winter wheat (Yamaguchi-Shinozaki, 2006). Moreover the studies on Arabidopsis mutants displaying reduced tonoplast  $\text{Ca}^{2+}/\text{H}^+$  antiport (CAX1) activity indicate that CAX1 participates in the development of the cold acclimation response (Lecourieux et al., 2006). On the other field of temperature acclimation, there exists data showing in Arabidopsis,  $\text{Ca}^{2+}$ /CaM have gotten involved in heat shock response (Zhang et al., 2009). And we also find some researches on overexpression of a CDPK in rice which brings increased tolerance to cold and salt stress (Saijo, Y. et al., 2000).

Taken together, depending on the type of signal or the type of cell, internal and/or external  $\text{Ca}^{2+}$  stores could be involved in raising  $[\text{Ca}^{2+}]_{\text{cyt}}$  (Dodd et al., 2010; Kudla et al., 2010). Both types of  $\text{Ca}^{2+}$  transporters, namely,  $\text{Ca}^{2+}$ -ATPases and CAXs involve in plant responses by regulating  $[\text{Ca}^{2+}]_{\text{cyt}}$ . Based on that, regulating cellular and intercellular  $\text{Ca}^{2+}$  signaling networks brings improving resistances or tolerances. And seeing from the regulation networks of stress responses to drought, salt and cold stress, we find  $\text{Ca}^{2+}$  and its interacting proteins may be the cross-talks among ABA-dependent, MAPK and other stress signaling pathways. Anyway, we can see the core of  $\text{Ca}^{2+}$  actions in relaying abiotic stress signaling depends on how to translate  $\text{Ca}^{2+}$  signatures to specific protein phosphorylation cascades, which we have mentioned above, so in the following part, we are going to trace the performance of phosphoproteins in signal transduction.

## 2.4. Role of phosphoproteins in stress signaling

By controlling the phosphorylation status of other proteins, protein kinases and phosphatases play a fundamental role in coordinating the activity of many known signal transduction pathways. For many signal pathways not only in abiotic stress field, protein reversible phosphorylation is the major player in relaying signals. And during this significant process, we highlight the functions of protein kinases and protein phosphatases who are enzymes to catalyze these reversible phosphorylation processes. And they are divided into several categories according to their structure or functional characteristics. And in the following part, we will give a general idea to the readers about their central role in signal transduction in abiotic stress aspect.

### 2.4.1. MAPK

Obviously, we have seen MAPK many times in formal description in this chapter. Even if it is not found in plants, the mitogen activated protein kinase (MAPK) cascades are known to be involved in plant abiotic stress responses acting as intracellular signal modules that mediate signal transduction from the cell surface to the nucleus. The reason to mention it here is that phosphorylation plays a central role in the progression of the signal through the MAPK cascade. Moreover MAPK cascades, the conserved signaling modules found in all eukaryotes, are fundamental in transducing environmental and developmental cues into intracellular responses bringing changes in cellular organization or gene expression. The simplest constitution of a MAPK cascade contains MAP kinase kinase kinases (MAP3Ks/MAPKKKs/MEKKs), MAP kinase kinases (MAP2Ks/MAPKKs/MEKs/MKKs) and MAP kinases (MAPKs/MPKs) (Mishra NS et al., 2006). And when under stress, stimulated plasma membrane will activate MAP3Ks or MAP kinase kinase kinase kinases (MAP4Ks), who may be the adapters to link upstream signaling steps to the core MAPK cascades (Dan I et al., 2001). Following that, MAP3Ks will phosphorylate two amino acids in the S/T-X3-5-S/T motif of the MAP2K activation loop. Then MAP2Ks phosphorylate MAPKs on threonine and tyrosine residues at a conserved T-X-Y motif at the active site. When signals come to MAPKs, further phosphorylation will tag on a wide range of substrates involving other kinases, cytoskeleton-associated proteins, and/or transcription factors. As for formation and integrity of a specific MAPK cascade, scaffold proteins take control over it (Whitmarsh AJ et al., 1998). And after signaling completed, MKPs (MAPK phosphatases) take the responsibility to shut the pathway down. Generally, the whole cascade is regulated by various mechanisms, including not only transcriptional and translational regulation but through post-transcriptional regulation such as protein-protein interactions (Rodriguez MC et al., 2010).

Thanks to traditional genetic and biochemical methods and lots of excellent research efforts, we can conclude that MAP3K/MAP2K/MAPK signaling modules show overlapping roles in controlling diverse cellular functions by forming complex interconnected networks within cells. These include cell division, development, hormone signaling and synthesis, and response to abiotic stress (high and low temperature, drought and high and low osmolarity,

wounding, high salinity, UV radiation, ozone, ROS, heavy metals), as well as biotic stress reactions (Jonak C et al., 2002; Xiong L et al., 2003; Raman, M. et al., 2007; Gohar Taj et al., 2010; Alok Krishna Sinha et al., 2011).

According to the researches on MAPK pathways, we can see that regulated expression of MAPK components shows effects on stress sensitivity. Here are some examples. Expression of an active form of a tobacco MAP3K, NPK1, increases freezing tolerance of transgenic tobacco or maize plants (Kovtun, Y et al., 2000; Shou, H et al., 2004). Meanwhile, MAP2K1 shows transcriptional induction under salt stress, drought and cold, as well as activated by wounding and drought stress. And MAP2K1 can phosphorylate MAPK4. An unsurprised fact is that MAPK4 and MAPK6 are found to be activated by cold, salt and drought (Ichimura K et al., 2000). Indeed, a MAPK module composed of MAP3K1-MAP2K1/MAP2K2-MAPK4/MAPK6 has been confirmed in cold and salt stress by yeast two hybrid analyses and yeast complementation (Teige M et al., 2004). So we can say different MAPKs are activated at different times after the onset of stress and the activities of these MAPKs are activated within different time periods. By the way, during osmolarity signaling MAPKs module seems to be widely involved (reviewed by Gohar Taj et al., 2010).

Due to the interlink between osmotic stress and oxidative stress, we are informed of the relationship between ROS, hormone signaling and MAPKs. ROS like  $H_2O_2$  is closely associated to MAPKs' activities. In Arabidopsis,  $H_2O_2$  activates AtMPK6 and the related AtMPK3 via the MAP3K ANP1 (Desikan R et al., 1999), and AtMPK6 are involved in cold stress as we knew before. Additionally, in tobacco, under  $H_2O_2$  and ozone treatment, the ortholog of AtMPK, SIPK1 will be activated as well (Samuel MA et al., 2000). These findings imply that multiple MAPK modules mediate oxidative stress responses and that MAPK cascades are not only induced by ROS but may also regulate ROS levels. Meanwhile, activating SIPK1 (salicylic acid-induced protein kinase), who is an NO-activated protein kinase in tobacco, can not process without SA, which brings a suggestion for the existence of cross-talk between ROS, hormone signaling and MAPKs. Here is some other evidence coming from studies on stomatal movement (Eckardt NA., 2009). In guard cells of *Vicia faba*, MAP2K is believed to regulate stomatal movement through mediating  $H_2O_2$  generation induced by ABA (Song XG et al., 2008). Later, in guard cells studies, MAPK9 and MAPK12 have been proved to serve as positive regulators acting downstream of reactive oxygen species and calcium signaling in ABA signaling. In 2.2.4, we talk about ABA- and  $Ca^{2+}$ -induced stomatal closure, so we can't help wonder is there any link between MAPK and ABA- and  $Ca^{2+}$ -induced pathways? Yes, it has been found that ABA and  $Ca^{2+}$  signals cannot activate anion channels in mpk9/12 mutants, thus indicating that these two MAPKs act between the ABA and  $Ca^{2+}$  signals and the anion channels. (Jammes F et al., 2009).

Even if the role of MAPK in ABA signaling has not yet been directly confirmed, the attempts to clarify the relationship between hormone and MAPKs never stop. Not just for ABA, the role of MAPK signaling cascades in auxin signaling and ethylene biosynthesis has been documented in numerous studies (Dai Y et al., 2006; Xu J et al., 2008). Eventually, the genes in hormone biosynthesis (ethylene) and responses (auxin) are altered, it is of great significance to distinguish the direct targets of MAPK cascade from those that are regulated

by altered hormonal and oxidative stress responses. On the other hand, recently in heavy metal stress studies, we find data supporting that MAPK3 and MAPK6 are activated responding to cadmium through ROS accumulation produced by oxidative stress in *Arabidopsis* (Liu XM et al., 2010), which further demonstrate the close relevance between ROS and MAPKs.

Meanwhile, by searching for the linkage between MAPKs and its various substrates involving other kinases and transcription factors, we gain much more information about MAPK cascades. Over-expression of MAP2K2 affects genes for several transcription factors (such as RAV1, STZ, ZAT10, ERF6, WRKY, and CBF2), disease resistance proteins, cell wall related proteins, enzymes involved in some secondary metabolisms and an 1-aminocyclopropane-1-carboxylic acid synthase (ACS). In the case of ACS, the rate-limiting enzyme of ethylene biosynthesis, the phosphorylation by MAPKs and by CDPKs affects protein stability and turnover, which again shows us the complicated cross-talk network (Bernhard Wurzinger et al., 2011). So it is a big challenge to identifying the targets of MAPK cascades, but the researches on other protein kinases leave us useful clue to find the answer.

#### 2.4.2. Other protein kinases

Whether at the transcript level or activity level, protein kinases are induced by a variety of abiotic stress, which indicates their powerful participation in signaling process. Moreover no matter suppressing or overexpressing these kinases, there both exists data showing that in transgenic plants, stress responses has changed. So far, we know several protein kinases involved in stress tolerance are stimulated by ABA, such as most of SNF1-related kinases (SnRKs) like SnRK2, SnRK3 (CIPK), CDPK and MAPK families. But others like Glycogen synthase kinase 3 (GSK3) (Jonak and Hirt, 2002; Koh et al., 2007), S6 kinase (S6K) (Mahfouz et al., 2006), SERK (Marcelo O. Santos et al., 2009) also attract a lot of attention.

Now let's start from SnRKs. The SnRK2 family members are plant-specific kinases relating to abiotic stresses responses and abscisic acid (ABA)-dependent plant development. They have been classed into three groups; members of group 1 are not activated by ABA, and group 2 also will not be activated or weakly activated, while group 3 is strongly activated by ABA. In *Arabidopsis* the SnRK2 subfamily consists of 10 members. Except SnRK2.9, all SnRK2s are activated by osmotic and salt stress (Boudsocq, M. et al., 2004). Take SnRK2.6 (OST1) as an example. SnRK2.6 functions in the ABA signaling pathway upstream ABA-induced ROS production. It is related to the ABA-activated protein kinase AAPK in *Vicia faba* and also associates with SNF1 protein kinase. NADPH oxidases function in ABA signal transduction, also targeted by the SnRK2.6 kinase (Nakashima et al., 2010). Generally speaking, regulating the response to ABA through SnRK2s pathways is to directly phosphorylate various downstream targets such as ion channels (SLAC1, KAT1) and ABFs and other specific TFs required for expression of stress-responsive genes (Anna Kulik et al., 2011). By the way, the SnRK2 subfamily is conserved in land plants. No wonder their role in ABA signaling and osmotic stress responses have also been found in pea, barley, rice and *zea mays* (Shen, Q. et al., 2001; Kobayashi et al., 2004; Huai, J. et al., 2008). As for SnRK3 (CIPK),

we compact it here that CIPK1/3/8/14/15/20/23/24 take part in ABA signaling. CIPKs play a main role in plant ion homeostasis and abiotic stress tolerance by regulating  $H^+$ ,  $Na^+$ ,  $Ca^{2+}$  and  $NO_3^-$  transporters and  $K^+$  channels and interacting with TFs (Kudla, J. et al., 2010). Moreover, the CDPKs that are involved in ABA signaling are CPK3/4/6/11/32. CPK4 and CPK11 are closely related genes and both phosphorylate the transcription factors ABF1 and ABF4. Based on a majority of evidence, it can be concluded that CDPKs target core ABA signaling components (Geiger, et al., 2010). A role for MAPKs in ABA signaling has been shown above, but posted here again (MPK1/2/3/6/9/12). Taken together, none of these protein kinase families function specifically in ABA signaling, which still need us to stress on functional redundancy and complicated cross-talk network in the future research.

As follows, we take a brief look at other kinases. CDKs (Cyclin dependent protein kinases) are a large family of serine/threonine protein kinases and mainly function in ensuring that cells progress in order over the different cell division stages. But their roles in abiotic stress responses turn out to be more eye-catching, which can be reflected in heat, cold, drought and salt stimuli researches (reviewed by Georgios Kitsios, 2011). Other findings like Somatic Embryogenesis Receptor Kinase (SERK) relating to somatic embryogenesis and apomixis (Marcelo O. Santos et al., 2009), AtNEK6, a member of the NIMA (never in mitosis A)-related kinases (NEKs) (Lee SJ et al., 2010), bring us a large amount of information to explore the functional importance of various kinases in abiotic stress.

#### 2.4.3. Protein phosphatases

During phosphorylation process, the job for protein phosphatases is to remove the phosphate added by protein kinases. Based on their substrate specificity, protein phosphatases can be classified, at least three families, as PPP family and PPM family composed by serine/threonine phosphatases, PTP family comprising tyrosine phosphatases, and dual specificity phosphatases (dsPTPs/dsPPase). As the largest group of phosphatases in plants, serine/threonine phosphatases can be further divided into PP1, PP2A, PP2B, and PP2C. For stress signal transduction, involvement of PP2C, PP2A, PTP, dsPPase have been reported in ABA or stress signal transduction, but the best-known example is the PP2C. On the other hand, many experiments have found that the relationship between MAPKs and phosphatases both exists in animals and plants. It has been shown that tyrosine-specific phosphorylation is associated with plant MAPKs, which again demonstrate the essential position phosphatases take in signaling pathways.

For the biggest branch of protein phosphatases in plants, in Arabidopsis alone, 76 PP2C genes have been identified early (Kerk et al. 2002). Further researches say ABA and other abiotic stress stimuli can induce A-type PP2C expression. Furthermore the A-type PP2C phosphatases, ABI1, ABI2, HAB1 (P2CHA) and PP2CA (AHG3) have been proved to directly interact with PYR/RCAR ABA receptors, however they always act as negative regulators at different layers of ABA signaling. (Merlot et al., 2001; Umezawa et al., 2009; Nishimura et al., 2010).

Working on other phosphatases like PP1, we find data in *Vicia faba* studies supporting its involvement in stomatal opening during the response to blue light (Takemiya et al. 2006). As to PP2A, five genes encoding its catalytic subunit has been identified in rice, and three of them show expression alteration under abiotic stress (Yu et al. 2003, 2005). And the activity of PP2B needs the help from calcium (Luan 2003). Some others like DSP4, a dual-specificity phosphatase, has been demonstrated to bind starch and interact with AKIN11, a SNF 1-related kinase in Arabidopsis (Fordham-Skelton et al. 2002; Kerk et al. 2006). Anyway we do need more facts to clarify the function network between phosphatases and other signals like hormone, ion channels, kinases actions.

Finally, we can see that the biggest challenge for abiotic stress signal research is to elaborate the network of kinases and phosphatases, and their relationship with a wide range of substrates, as well as understand the phosphorylation states and how phosphorylation-dependent activity culminates in the process of coping with a particular environmental stress.

## 2.5. The role of TFs and genes in certain abiotic stress situations

By the end of signaling, the biggest assignment is to temporally and spatially regulate stress-induced genes expression, and it is almost done at transcriptional level (Rushton and Somssich 1998). Obviously the most contributing workers are transcription factors (TFs) who modulate genes expression by binding to specific DNA sequences in the promoters of target genes (Chaves and Oliveira 2004). Thanks to coordination between TFs and genes, new transcripts are synthesized and stress adaptations have been realized within just a few hours. Consequently, that is why TFs are such a group of powerful targets being so popular in genetic engineering field aiming at improving stress resistance in crop plants. But, who are they? While, most of them belong to several big families, naming AP2/ERF (ethylene responsive element binding factor), Zn finger, basic leucine zipper (bZIP), basic helix-loop-helix (bHLH), WRKY, MYB, and NAC. Indeed we frequently mentioned them in formal description. So let's start to wander in their complicated regulatory network designed for the also complex stress situations.

### 2.5.1. Drought

Based on recent data, the rate of land area experiencing drought is uprising and probably goes up to 30% by the end of this century (Yi et al. 2010). And that is a big threaten for plants lives, for drought is very likely to give rise to arrest of photosynthesis, disturbance of metabolism and finally plant death (Jaleel et al. 2008). But plants will react efficiently. With a few second of water loss, phosphorylation status of a protein will be triggered and later when the suffering time reached hours or days long, gene expression and plant morphology occur (Verslues and Bray, 2006). By researching on Arabidopsis plants under water-deficit stress, more than 800 induced genes have been identified (Bray, 2004), who play key roles in signal transduction, transcriptional regulation, cellular metabolism and transport, as well as cellular structures protection. Meanwhile, when water deficit comes two major

transcriptional regulatory pathways of gene expression show up. One group is TFs called dehydration response element binding protein (DREB) coming from AP2/ERF family and another is NAC, AREB/ABF, WRKY and MYB group. The former work in the ABA-independent pathway, latter known to be as the most responsive to ABA signaling under drought.

The DREB proteins contain an ERF/AP2 DNA-binding domain that is quite conserved. The TFs containing it are widely found in many plants, including Arabidopsis (Okamuro et al. 1997), tomato (Zhou et al. 1997), tobacco (Ohme-Takagi and Shinshi 1995), rice (Weigel 1995) and maize (Moose and Sisco 1996). And a conserved Ser/Thr-rich region next to ERF/AP2 domain is considered to be responsible for DREB proteins phosphorylation (Liu et al., 1998). Latter, Agarwal et al. found high sequence similarity exists in different DREB proteins by amino acid alignment analysis (Agarwal et al., 2006).

Generally, The DREB TFs could be divided into DREB1 and DREB2, and they participate in signal transduction pathways under low temperature and dehydration respectively. They belong to plants-distinctive ERF family. ERF proteins share a conserved domain binding to the C-repeat CRT/dehydration responsive element (DRE) motif engaged in the expression of cold and dehydration responsive genes (Agarwal et al., 2006). The expression of DREB2A and its homolog DREB2B are induced by dehydration and high salt stress (Liu et al., 1998; Nakashima et al., 2000). Furthermore, the expression of DREB genes is induced by abiotic stresses at different time periods (Liu et al. 1998; Dubouzet et al. 2003). But for the character of tissue-specific expression, the data is still in short.

For the ABA-dependent gene induction under water-deficit condition, we want to stress on another group of TFs. Firstly, MYB, MYC, homeodomain TFs and a family of transcriptional repressors (Cys2/His2-type zinc-finger proteins) are involved in the ABA response to water deficit. The promoter region of Responsive to Dehydration 22 (RD22), who is induced by ABA, contains MYC and MYB *cis*-element recognition sites. And MYC and MYB TFs only accumulate after an increase of ABA concentration, and Over-expressing these TFs lead to promoted ABA sensitivity and drought tolerance (Abe et al. 2003). Also other data indicate that two R2R3-MYB TFs (AtMYB60 and AtMYB61) are directly involved in stomatal dynamics in Arabidopsis regulated by light conditions, ABA and water stress (Liang et al. 2005). Very recently, in Arabidopsis, it has been strongly suggested that WRKY TFs possibly act downstream of at least two ABA receptors, the cytoplasmic PYR/PYL/RCAR protein phosphatase 2C-ABA complex and the chloroplast envelope-located ABAR-ABA complex. And the promoter-binding experiments show that the target genes for WRKY TFs involved in ABA signalling include *ABF2/4*, *ABI4/5*, *MYB2*, *DREB1a/2a*, *RAB18*, *RD29A* and *COR47*. Other findings in a large sense prove us WRKY TFs are positive regulators of ABA-mediated stomatal closure being relevant with drought responses (reviewed by Deena L. Rushton et al., 2011). On the other hand, it has been realized earlier that the ABA response element (ABRE) is bound by basic Leucine Zipper Domain (bZIP-type) TFs, and three Arabidopsis bZIP TFs (AREB1/ABF2, AREB2/ABF4, and ABF3) are activated through phosphorylation reacting to water deficit and ABA treatment. Other NAC domain proteins ANAC019, ANAC055, and ANAC072 are also induced by the same treatment. And in guard

cells, it has been shown that the strong induction of Stress Responsive–NAC1 (SNAC1) gene expression by drought also affect stomatal closure (Hu et al. 2006).

### 2.5.2. Flooding stress

Flooding and submergence are two stresses lead to anoxic conditions in the root system. Under this stress condition, both anoxia and hypoxia are defined by O<sub>2</sub> shortage. But diverse plants have their own way to adjust to it. Some lowland rice cultivars, such as FR13A, can survive submergence by suppressing shoot elongation. At the molecular level, Submergence-1 (Sub1), which is derived from FR13A, is a major quantitative trait locus contributing to great submergence tolerance (Xu et al. 2006). And three sequentially arrayed genes (designated *Sub1A*, *Sub1B*, and *Sub1C*) has been identified. *Sub1A* has been proved to encode an ERF domain-containing TF associated with the induction of low oxygen escape syndrome (LOES) (Bailey-Serres and Voesenek, 2008 ).

In plants, different cell types exhibit a conserved response to low oxygen levels at the molecular level (Mustroph et al. 2010). This response includes the induction of genes after 30 min under hypoxia, whose expression is maintained for several hours (Klok et al. 2002; van Dongen et al. 2009). The increased transcript levels of these genes are further accompanied by active combination between mRNAs and polysomes reflecting promoted translation process (Branco-Price et al. 2008). Unfortunately, in plants the mechanism by which oxygen is perceived has not been clarified. But researches on hypoxia-responsive TFs can help us a lot to investigate the regulation of the hypoxic response. Usually these TFs are detected in families like MYB, NACs [Arabidopsis Transcription Activation Factor (ATAF) and Cup-shaped Cotyledons (CUC)], Plant Homeodomain (PHD) and ERF families (Hoeren et al. 1998; Christianson et al. 2009; Licausi et al. 2010b). And on the other hand, Microarray data in Arabidopsis and rice research find us several transcription factors whose expression increases induced by oxygen deprivation, such as heat shock factors, MADS-box proteins, and WRKY factors (Lasanthi-Kudahettige et al., 2007). Recently Licausi et al. (2010a) have identified TFs that are differentially expressed under hypoxic conditions. The results indicate members of the AP2 / ERF-type family are the most common upregulated TFs, followed by Zinc-finger and basic helix-loop-helix (bHLH-type) TFs. TFs belonging to the bHLH family also appear in the downregulated part, together with members from the bZIP and MYB families.

On the other hand, by *silico* experiments and trans-activation assays it has been confirmed that five hypoxia-induced TFs (At4g29190; LBD41, At3g02550; HRE1, At1g72360; At1g69570; At5g66980) from different TF families [Zinc Finger, Ligand Binding Domain (LBD)/Lateral Organ Boundary Domain, ERF, DNA binding with one finger (DOF), ARF] respectively showed the ability to regulate the expression of hypoxia responsive genes (Licausi et al. 2010b). Other evidence relating to TFs and adaptive response to low oxygen will refer to redox-sensitive TFs. ZAT12, a putative zinc finger-containing TF, is identified as an important link in the oxidative stress response signalling network in Arabidopsis (Rizhsky et al. 2004), for its transcript levels were remarkably mounted up in response to hypoxia and anoxia in several independent analyses (Branco-Price et al. 2005).

### 2.5.3. Salinity

Plants growing in high salt concentrations, as we know, will suffer from osmotic stress and take actions like closing stomata and reducing cell expansion in young leaves and root tips. Subsequently, accumulation of ions, especially sodium ( $\text{Na}^+$ ), in the photosynthetic tissues, will hit photosynthetic components such as enzymes, chlorophylls, and carotenoids (Davenport et al. 2005), followed by secondary stresses (oxidative stress and nutritional disorders) (Hasegawa et al. 2000; Chinnusamy et al. 2006).

One of the main strategies taken to improve plant salt tolerance is to re-establish ion homeostasis by counteracting the osmotic component of the stress to avoid toxic concentrations within the cytoplasm (Munns and Tester 2008). Recently, in *Arabidopsis* it has been confirmed that the effective establishing and maintaining ion homeostasis is mediated mainly by a Salt Overly Sensitive (SOS) signal pathway, which we refer to in  $\text{Ca}^{2+}$  part. Recently, SOS4 and SOS5 have also been characterized (Mahajan et al. 2008). Similar to *SOS1*, *Arginine Vasopressin1 (AVP1)* and *A. thaliana Na<sup>+</sup>/H<sup>+</sup> exchanger1 (AtNHX1)* genes contribute to ion homeostasis also (Gaxiola et al. 2001; Zhang et al. 2001). Besides these genes, overexpression of genes encoding LEA proteins, such as the barley HVA1 (Xu et al. 1996) and wheat dehydrin-5 (DHN-5) (Brini et al. 2007), is confirmed to be able to enhance plant salt tolerance. And regulating *Lea* gene expression is mediated by both ABA dependent and independent signalling pathways, both of which use  $\text{Ca}^{2+}$  signaling to induce *Lea* gene expression during salinity.

Meanwhile, what do TFs do in salt stress signaling? Based on researches on *Cor/Lea* salinity stress responsive genes, whose expression is mediated  $\text{Ca}^{2+}$  and ABA in salt stress signaling, it has been indicated that various upstream TFs will activate DRE/CRT, ABREs, MYC recognition sequence (MYCRS) and MYB recognition sequence (MYBRS) *cis*-elements. Also, with or without the involvement of ABA makes them differs from each other. On one hand, ABRE and MYB/MYC element-controlled gene expression is ABA-dependent, which activates bZIP TFs called AREB binding to ABRE element to induce the stress responsive gene (*RD29A*). However, even if ABA makes these TFs function in their own regulating ways, it has been shown that ABA-dependent and -independent TFs may also cross talk to each other in a synergistic way to amplify the response and improve stress tolerance (reviewed by Dortje Golldack et al., 2011).

### 2.5.4. Extremes of temperature

Signals of extreme temperature, from freezing to scorching, is perceived by membrane and transduced by different transduction components results in transcription of several genes. Cold stress directly inhibit metabolic reactions and indirectly produce harm through cold-induced osmotic prevents the expression of full genetic potential of plants owing to its direct inhibition of metabolic reactions and, indirectly, through cold-induced osmotic, oxidative and other stresses such as water uptake barriers caused by chilling and cellular dehydration induced by freeze. Cold stress, based on temperature range, are defined as chilling ( $<20^{\circ}\text{C}$ ) and/or freezing ( $<0^{\circ}\text{C}$ ) temperatures, both of which hurt plants in different ways. The

former leads to slow biochemical reactions related to enzymes and membrane transport activities, while the latter forming ice crystal can cause membrane system disruption (Chinnusamy et al. 2007).

Numerous TFs in cold stress circumstances have been identified in *Arabidopsis*, homologs of which have been reported in other plants also. Significant progress has been made in the past decade in elucidating the transcriptional networks regulating cold acclimation. Firstly, AP2/ERF family TFs, CBFs play essential role in controlling genes in phosphoinositide metabolism, osmolyte biosynthesis, ROS detoxification, hormone metabolism and signalling (Lee et al. 2005). They can bind to *cis*-elements in the promoters of COR genes to activate gene expression. Earlier it was proved that *DREB1A/CBF3*, *DREB1B/CBF1* and *DREB1C/CBF2* genes, lying tandemly in the *Arabidopsis* genome, are induced by cold but not by dehydration or high salinity (Shinwari et al. 1998). *CBF* genes showed high expression under low temperature treatment and the transcript was detectable after 30 min exposed to 4 °C and reached maximum expression at 1 h (Medina et al. 1999). However this time-linking phenomenon differs from various plants. In rice, detecting *OsDREB1A* and *OsDREB1B* transcript might need to wait 40 min after cold exposure. By the way, *CBF* pathway might also have a crucial role in constitutive freezing tolerance (Hannah et al. 2006).

Moreover for many studies, emphasize has been laid on the ICE-CBF-COR transcriptional cascade. In *Arabidopsis*, ICE1 (Inducer of CBF Expression1), a MYC-type bHLH TF, can bind to MYC recognition elements in the *CBF3* promoter affecting its expression during cold acclimation (Chinnusamy et al. 2003). Besides being the inducer of *CBFs* transcription, ICE1 is also a transcriptional inducer of *ZAT12*, *NAC072* and *HOS9* in *Arabidopsis* (Benedict et al. 2006). Furthermore, by studying on *ice1* mutation under cold stress, the genes in calcium signaling, lipid signaling or encoding receptor-like protein kinases are found to be affected (Lee et al., 2005). In conclusion, there do exists network between these components in cold signaling. Constitutive expressed ICE1 is activated through sumoylation and phosphorylation induced by cold stress, which then induce the transcription of *CBFs* and reprime *MYB15*. For *CBFs* expression, *CBF2* acts as a negative regulator of *CBF1* and *CBF3* expression. Meanwhile, the expression of *CBFs* is negatively regulated by upstream TF *MYB15* and *ZAT12*(Chinnusamy et al. 2007).

### 3. Conclusion

Thanks to so much efforts made by researchers in various fields, we have a pretty clear idea about how to develop our researching methods in abiotic stress field. That is exactly what we should concentrate on in the future. For plants' reactions to different kinds of stress, we must make more efforts in taking measures in focusing on systematic studies which so far can be taken as the best way to figure out what plants will do under certain circumstances.

As we all know, it is of incredibly great importance to understand more about abiotic stress which impacts a lot on plants, which will not only change our understanding of current environment we live, but also bring a plenty of benefits for improving human beings living

standards. That is why at this part we hope we can get everyone's attention to how to explore plants kingdom and develop researches in a systematic way.

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